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**AMRL-TDR-62-142** 

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### A STUDY OF MICROBIOLOGICAL WASTE TREATMENT TECHNIQUES

TECHNICAL DOCUMENTARY REPORT NO. AMRL-TDR-62-142

November 1962

Biomedical Laboratory
6570th Aerospace Medical Research Laboratories
Aerospace Medical Division
Air Force Systems Command
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[Prepared under Contract No. AF 33(616)-8153 by Frederick W. Bieberdorf Southwest Research Institute, San Antonio 6, Texas]

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#### FOREWORD

This report was prepared by Southwest Research Institute under AF Contract No. AF 33(616)-8153 entitled "A Study of Microbiological Waste Treatment Techniques," Project No. 7164, "Space Biology Research," and Task No. 716403, "Environmental Biology."

This contract was initiated by Richard E. Bennett, Respiratory Equipment Section, Sustenance Branch, Life Support Systems Laboratory, 6570th Aerospace Medical Research Laboratories. Responsibility for this contract was later transferred to Biospecialties Section, Physiology Branch, Biomedical Laboratory. Dr. Sheldon A. London is contract monitor. This study was begun in March 1961 and completed in March 1962.

#### ABSTRACT

A number of chemical and microbiological methods for the disposal of human wastes have been surveyed. On space flights of moderate duration (ca 100 to 1000 days) it would be most practical to treat the wastes with biocides and store them after dehydration for recovery of the water. On longer flights the wastes either must be chemically or microbiologically oxidized so that the carbon and chemically bound water could be recovered and used in photosynthesis. Chemical oxidation is more rapid and complete but requires the use of elaborate equipment and the possible expenditure of some heat energy. Microbiological degradation is slower but takes place at a lower temperature. When used in conjunction with a photosynthetic step, this might be the best solution to the problem for use of lunar colonies or prolonged space voyages. A procedure is suggested by which algal and bacterial colonies separated by semipermeable membranes could be used for waste digestion and reutilization of the carbon. An alternative to this procedure is suggested in which methane-utilizing bacteria and algae are used symbiotically for the conversion of wastes to protoplasm, with a minimum expenditure of energy.

#### PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

SOS. M. QUASHNOCK Colonel, USAF, MC

Chief, Biomedical Laboratory

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#### I. INTRODUCTION

During short orbital or circumlunar journeys into space, it will probably be satisfactory to store solid human wastes in hermetically sealed containers or jettison them. However, when permanent space stations, lunar colonies, and interplanetary travel become possible, disposal of wastes in this manner will no longer be economical. It will be necessary to recycle the oxidized carbon produced by respiration and contained in human wastes in order to provide life support systems for long voyages. Estimates presently range up to \$1,000 per pound of payload put into orbit. Even if this decreases with the advent of more efficient rocket motors, a crossover point ultimately will occur at which recycling of oxygen and carbon becomes necessary. It is now estimated that chemical recovery of oxygen from CO<sub>2</sub> will be required in missions lasting over 20 days, and recycling of carbon in a photosynthetic gas exchanger will be necessary beyond 1,000 days.

The Apollo spacecraft is expected to make a round trip to the moon carrying three men by 1970. The duration of this mission is estimated to be about 14 days. This may be followed up by the establishment of a permanent orbiting satellite from which deep space probes may be launched. This would carry a complement of 15 men and would remain in space indefinitely. Alternatively, a permanent lunar base will be established for the same purpose. Therefore, it is evident that life support systems

in which carbon and oxygen can be recycled will be required within the next 15 years.

This report summarizes some of the principal methods by which human waste could be introduced into such a system. These include chemical oxidation, oxidation by aerobic bacteria and fermentation by anaerobic bacteria. It is believed that any of these processes would have to be incorated in a cycle containing a photosynthetic step to make the operation feasible.

#### II. NATURE AND COMPOSITION OF WASTES

The wastes which must be disposed of in space capsules include feces, urine, volatile compounds, wash water and perhaps cleansing materials and left-over foods. It can be assumed that these latter materials could be reduced to a minimum in a well-organized life support system. These wastes would contain some of the components found in domestic sewage. However, they would differ markedly from sewage particularly with regard to lower contents of cellulose and lignin.

#### A. Feces

The average adult produces 80 to 120 grams of feces daily (ref. 13 p. 219, 6), so it is anticipated that a waste disposal unit designed for one to six astronauts would have to handle an average load of from 80 grams to 720 grams daily depending on the size of the crew. It is likely that this figure will be reduced somewhat because of specialized diets that will be designed for space use, and possible use of hypothermia and other methods to reduce metabolic rates on prolonged space voyages. It is not anticipated that radical changes in waste digester design would be required for space missions involving one to six crew members. However, as the number of crew members increased greatly above this figure, additional facilities would probably be required. It is anticipated that units with different holding capacities would have to be designed for each type of mission.

Considerable effort has been devoted to studies on composition of human waste (ref. 7, 8, 9, 12). It has been observed that considerable variations exist. Goldblith and Wick (reference 6, page 25) conclude that "in view of the complex biochemical system existing in feces and the inherent difficulty encountered in isolation of their components it is suggested that any further investigation should be carried out on feces resulting from authentic space diets of the type that persons on flights of long duration would eat. If this were done a relatively more realistic idea of the nature of the available feedli components could be obtained and better criteria for their usefulness could be devised."

However, if the wastes are degraded microbiologically rather than using them directly as a source of nutrients, their exact composition is unimportant providing they contain all of the basic nutrients required to support microbial digestion and resynthesis of foods by algae or higher plants. The occurrence of essential amino acids and specialized cofactors is unimportant if it is assumed that the microorganisms which will be used in waste regeneration are essentially autotrophic. The appearance of specific organic compounds in the waste will be of importance primarily from the standpoint of toxicity. Other materials which will be of interest will be those which are not readily degraded by most microorganisms such as keratin.

Feces are reported to contain 60-70% water (ref. 6, 24). About 25-50% of the solids are made up of living and non-living bacteria (ref. 1, 6). In addition to bacteria, feces also contain mucous from the

intestinal mucosa, undigested cellulose fibers from fruits and vegetables, undigested food, and biopigments. A compilation of the major components of human feces is shown in Table 1. It should be noted that only 89% of the total material is accounted for, and much of the solid content has not been identified. Of the known groups of organic compounds, lipids occur most abundantly, comprising about 3% of the total weight. About 30% of the lipid fraction is unsaponifiable and consists mostly of cholesterol and its reduction products (ref. 6). Other lipids which are present in feces are neutral fats, fatty acids, and soaps. The individual fatty acids which have been identified include palmitic, stearic, oleic, myristic, lauric, and linoleic acids. Other lipid-like substances which occur in small quantities include cholic, deoxycholic and lithocholic acids (ref. 6).

Dried feces contain an average of 6% nitrogen on a dry weight basis. The nitrogen is contained in compounds such as indole, skatole, and many of the B vitamins, as well as bacterial proteins. Fecal proteins have been isolated in about 75% purity in yields of about 12% on a dry weight basis. (ref. 6). The protein has been hydrolized and the constituent amino acids identified. Only 22.6% of the amino acids of fecal protein are essential compared to a value of 48.1% for egg albumin. However, this observation is relatively unimportant if autotrophic microorganisms are used for waste disposal and regeneration.

Feces contain only small amounts of mono and oligosaccharides since only small amounts of these compounds are unabsorbed during digestion. Unabsorbed carbohydrates present in the feces include cellulose,

vegetable fibers, and pentosans. These will be important in the overall design of a microbiological disposal unit since their degradation by microorganisms is slow. The amounts of cellulose and pentosans present in feces are variable due to differences in diet. However, the content of these materials will probably be kept as low as possible in diets designed for astronauts. Minerals are of special interest since these are required for the growth of all microorganisms, however autotrophic these may be. Elements which are excreted in relatively large amounts are shown in Table 2. These include sodium, potassium, calcium, magnesium, chloride, phosphorous and sulfur. Other elements shown to be present include copper, manganese, iron and nickel. Data on boron have not been reported. This element is known to be essential to the growth of plants and might have to be supplied as a supplement if the residues from waste disposal are used to support photosynthesis.

The caloric value of feces is low. Thus, only 70 to 140 calories is excreted per man per day compared to an average intake of several thousand calories. However, this is not a disadvantage since efficient utilization of food by man is important from a health-efficiency standpoint. The important thing is to be able to recover the oxidized carbon and convert it to reduced forms which can be reused as foods.

#### B. Other Solid Wastes

Other solid wastes of potential importance include nail and hair clippings. These would be unimportant in short voyages but in interplanetary trips or permanent lunar colonies they might eventually create a

disposal problem from the standpoint of immobilized significant amounts of carbon and nitrogen. Keratin is one of the chief constituents of nails and hair. Very few organisms possess the ability to digest it enzymatically. The most notable of the organisms which can accomplish this are a group of fungi known as the dermatophytes. These cause skin disorders frequently referred to as ringworm. However, it is suspected that some airborne saprophytes like Alternaria, Scopulariopsis, Spondylocladium and certain actinomycetes may also be able to do this (ref. 11, 12). Therefore, it is possible that a search for specialized microorganisms would have to be made to find species which are capable of decomposing wastes which are not attacked by ordinary sludge bacteria.

#### C. Liquid Wastes

Liquid wastes will consist of the water contained in feces, wash water, and urine. It is anticipated that urine will be the most important of these from a volume standpoint. Urine contains from 92 to 97% water, urea and sodium chloride being the other chief ingredients (ref. 6, 9, 13). The average human excretes about 1,500 ml of urine during a 24 hour period. The constituents of normal urine are shown in Table 3. The principal organic compound contained in it is urea. It is the main end product of protein metabolism, and represents 80-90% of the urinary nitrogen. Other nitrogenous compounds present include ammonia, creatinine, uric acid, amino acids, hippuric acid, indican, allantoin, and creatine. All of these might conceivably supply nitrogen for the support of bacterial and algal growth.

Non-nitrogenous compounds present in urine include glucose, phenol, citric acid, and ascorbic acid. Essential minerals include iron, copper, magnesium, calcium, potassium, sulfur, etc. These are essential for the support of bacterial and algal growth. However, it should be noted that chloride, sodium, and potassium ions occur in large quantities. These can be toxic in high concentrations, so that considerable dilution of the urine may be required to obtain rapid growth of microorganisms.

Urine may have pH values as low as 4.6 and as high as 8.2 under extreme conditions (ref. 24). The mean pH of a 24-hour sample is generally about 6. The pH of the urine is dependent almost entirely upon the nature of the diet. High protein diets yield acidic materials while high vegetable and fruit diets yield basic residues because of the oxidation of the organic moieties of the potassium and sodium salts of the di- and tricarboxylic acids. The kidneys excrete acid phosphate, which is the chief factor in determining urine pH. Each species of microorganism has an optimum pH at which it will grow best. This optimum may vary from species to species, and the range may be quite wide in mixed cultures. However, sudden fluctuations in pH could change microbial balance and upset the operation of a waste disposal unit. Consequently, a control of pH throughout the process will undoubtedly be very important. Bacteria usually function best in a neutral or slightly alkaline environment, while fungi excrete organic acids and grow well in their presence.

#### D. Gas and Vapors

Gaseous wastes will include water vapor from respiration and perspiration, methane, hydrogen, hydrogen sulfide, and odorous materials not yet identified. Moreover, the average adult at rest absorbs and utilizes some 250 ml of oxygen per minute and eliminates about 200 ml of carbon dioxide. Methods for the regeneration of respiratory carbon dioxide will not be considered in this report, but of course must be included in the design of any completely self regenerating lite-support system.

Odorous gases and vapors may occur in the atmosphere of a waste disposal unit, or in the open atmosphere of a space capsule. They may be generated by normal metabolic processes such as breathing and perspiring, from the preparation of food, from the malfunction of equipment, or from the presence of materials from which the emission of vapors is unsuspected.

Hydrogen, methane, and hydrogen sulfide are present in flatus, which is discharged at a rate of 2 liters per day per person. Volatile compounds which have been identified in the atmosphere of a space cabin simulator are shown in Table 4. Some of these compounds such as propionaldehyde, isobutyraldehyde, various alcohols, and other organic compounds are probably of metabolic origin. Others such as the Freons and methyl chloride are probably derived from equipment used in the space cabin simulator. Many of these metabolic products would be formed in the waste digestion unit as well as being found in the atmosphere of the cabin. They may not be metabolized rapidly enough to permit satisfactory operation of a life

support system. Therefore, it is believed that it might be most convenient to dispose of them by catalytic combustion, and return the carbon dioxide and other gases formed to the photosynthetic unit for recycling. This might be accomplished in a unit containing Hopcalite. Units such as these are used for oxidizing carbon monoxide and hydrogen in the atmospheres of nuclear submarines. At low temperatures, hydrocarbons accumulate on the surface of the catalyst and interefere with its operation. At higher temperatures these are burned also. It is believed that such a step used in conjunction with a waste disposal unit would be an effective way to control the accumulation of noxious gases and vapors.

#### E. Nutrition Requirements

From the foregoing, it is evident that human wastes contain most of the major components essential to microbial metabolism. These include carbon, nitrogen, oxygen, phosphorous, potassium, iron, magnesium, calcium, and others. Certain essential micro nutrients such as boron have not been detected. Possibly these are present in the requisite amounts, or if not they could be added easily with a negligible increase in the payload required. The microorganisms used in a waste disposal unit would be autotrophic insofar as possible. Therefore, they would not be dependent for growth upon the presence of specific amino acids or co-factors. The presence of all the elements essential to growth in the correct ratios and in available forms would be all that would be required other than a source of energy. It is possible that human wastes would be low in phosphorus and

perhaps too high in other elements such as sodium and chloride ions.

These topics require further investigation.

The principal consideration in the design of a waste disposal unit would be to determine if the elements present in the waste would be readily available for use by microorganisms. So far, it has been shown that nitrogen and carbon contained in wastes can be metabolized by bacteria and reused by algae in the biosynthesis of protoplasm. However, more work needs to be done or studies of the recycling of inorganic nutrients.

#### III. ENVIRONMENTAL FACTORS

Any waste disposal unit proposed for use in a space cabin must be designed to function under specialized environmental conditions. These include reduced pressure, weightlessness, and possible exposure to high energy radiation from solar flares. Contamination of cultures and malfunctions which would interfere with the operation of the unit could have disastrous consequences. Also, it would be necessary to keep the power requirements as low as possible, and maintain the temperature close to the ambient temperature of the cabin.

#### A. Atmospheric Pressure

According to Stubbs (ref. 21) the minimum cabin pressure which should be used in trips of long duration should be 6.75 psia. This is eqivalent to an altitude of 20,000 feet. The oxygen content of the air in the cabin should be about 50%. Under these conditions the air would contain essentially equal amounts of oxygen and nitrogen. Stubbs (ref. 21) states that for prolonged flights the choice for the cabin atmosphere should undoubtedly be that enjoyed on the earth's surface, or close to one atmosphere. However, in experiments made in the space cabin simulator at the School of Aerospace Medicine, atmospheric pressures of 1/2 atmosphere were used in simulated flights of 17 and 30 days duration without ill effects (ref. 15).

There is no reason to believe that microorganisms would react adversely to this environment, particularly since the partial pressure

of oxygen would always be close to that at sea level. Moreover, the waste disposal unit would be isolated from the cabin atmosphere to prevent cross contamination. Therefore, it could be regulated at any desired pressure. For these reasons, it is unlikely that the pressures that would be used in space cabins would offer any serious problems in the design of a waste disposal unit.

#### B. Effects of Weightlessness

Present information indicates that short periods of weightlessness do not have profound effects on metabolism. Man has already been subjected to this stress for 24 hours, and no serious side effects have been reported. However, some evidence exists which indicates that unicellular organisms multiply more rapidly in the absence of gravity. Therefore, long term experiments in artificial earth satellites will be required before weightlessness can be dismissed as an unimportant factor in microbial growth. Although gravitational forces are comparatively small, earth bound life forms have evolved under this field for billions of years. Obviously, its removal for long periods of time could have serious effects on cellular growth and reproduction.

Moreover, several aspects of this condition would have to be considered from a mechanical viewpoint. Carbon dioxide generated by bacteria and oxygen released from water by algae would appear in the liquid matrices as minute gas bubbles. These might tend to move towards the surface very slowly because of the weightlessness of the liquid and gas phases. Eventually these bubbles would be forced from the liquid by internal cohesive forces, but this could be a slow process.

Other mechanical problems would be encountered in the transfer of liquids and the recovery of the final product of waste digestion and resynthesis. For example, removal of algae by precipitation or ordinary filtration would not be possible. Undoubtedly, pressure filters or some form of centrifugation would be required.

The form a liquid mass would take within a closed container under zero gravity would also present problems, but these could be compensated for by changing the interfacial tension between the liquid and the walls of the container. If the liquid holding the wastes did not wet the container, it would probably exist as a sphere suspended in air. However, if the container walls were hydrophilic, the liquid would probably adhere to them, and the air space would appear as a central bubble. Either of these conditions might be undesirable in operating a waste disposal unit. It is possible that this problem could be solved by constructing a container having both hydrophilic and hydrophobic walls so that the liquid within it could be positioned as desired.

Some preliminary designs have been made of a waste disposal unit for operation under conditions of weightlessness. In this design, it is proposed to force liquids from one chamber to another and to keep them under pressure by use of an inflatable bladder. It is anticipated that weightlessness in this context would be primarily an engineering problem, and would not influence the fundamental considerations that would be taken into account in designing a unit.

#### C. Contamination

Contamination of the bacterial cultures within the digester would present a serious problem that would require constant surveillance. For this reason, a careful study of the organisms making up the microbial flora of the unit is recommended. This is particularly important in the case of anaerobic digesters since pathogenic bacteria thrive under such environments. Consequently, great care should be taken that disease causing organisms are not carried into the space cabin by this means.

Also contamination of the cultures with organisms which produce toxic end products might upset the operation of the unit. This could also have disastrous consequences. Thus, the nature of the flora present in the unit will require careful study.

#### D. Radiation Hazards

#### 1. Radiation Sterilization

It is well known that exposing microorganisms to radiation may have lethal effects upon them. The mechanisms of action of the various types of radiation are not wholly understood; however, broadly speaking damage results either from direct hits on chromosomal material, or secondarily through the formation of free radicals. Types of radiation known to kill microorganisms fall into two groups: (a) electromagnetic waves comprising γ-rays, X-rays, and ultraviolet radiation, and (b) high velocity particulate radiation including α-particles, β-particles, high energy protons and deuterons, and nuclei of the heavier elements. The death rates of organisms under the influence of most lethal radiations

follow an exponential curve. In general, ED<sub>50</sub> values in radiation sterilization depend on the total dose received, and within limits are independent of the time of exposure. Most of the work done on the killing of bacteria with radiation has been carried out with γ-rays for the express purpose of sterilizing foods. Sykes (ref. 22) has included a comprehensive review of this topic in his book on disinfection and sterilization which is of value from the standpoint of obtaining an insight into the theoretical principles behind the process. However, which more needs to be known concerning the biological effects of the high energy protons produced by solar flares before the effects of exposure of microorganisms to the spacecraft environment can be fully assessed.

#### 2. Mutations

The occurrence of mutations in the bacterial flora of a waste disposal unit will be impossible to stop altogether, since they can occur spontaneously to a rare extent, and can be induced by chemicals. Shielding of the waste disposal unit by the walls of the spacecraft and possibly by the water carried on board would probably decrease mutation rates to acceptable values under normal conditions. However, complete shielding against solar flares would probably be impossible because of the added mass. The same will probably be true of the human occupants of the spacecraft unless some form of electromagnetic shielding against high energy protons is forthcoming.

Pending this development, the best solution appears to be the avoidance of space flight during periods of high solar activity, and/or

increasing the resistance of the organisms to radiation. This latter can be accomplished temporarily by reducing oxygen tension, or by use of anti-radiation chemicals. Novick and Szilard (ref. 18), for example, have shown that the number of mutations induced in bacteria and fungi by caffeine and theophylline can be reduced markedly by including the nucleoside guanosine in the culture medium.

More work using sounding rockets and satellites needs to be done to assess the effects of radiation during periods of high solar activity. Davis (ref. 5) has shown that heat stable clostridiospores were not killed when placed aboard a Discoverer Satellite. However, it must be presumed that continuous exposure to the Van Allen Belt and solar flares would produce both lethal and mutant effects.

To guard against the first possibility, it would be necessary to carry additional innocula in an inert state in which they could be heavily shielded with a minimum weight penalty. These could be used to reactivate the disposal unit in case of failure. The use of lyophilized bacteria would have advantages in this application since they would require little space and are resistant to radiation.

The complexity of the flora contained in anaerobic and aerobic digestors in itself may afford some protection against mutations. Bacterial mutants (viable) usually lack the ability to synthesize specific amino acids and/or cofactors. It is extremely unlikely that all of the bacteria present would undergo mutations that would produce the same deficiencies. Therefore, it is most likely that even mutant species would continue to grow

and participate in the dissimilation of wastes, since they could derive the compounds they were unable to synthesize from other microorganisms.

Mutants of this type are usually observed only in pure culture on a defined medium. Moreover, occassional lethal mutants would not be important because of the large number of the bacteria present, and the rapidity with which they multiply. Except under conditions of high solar activity, it is more likely that the microbial flora would become unbalanced by sudden changes in the composition or concentrations of the waste, than by the production of mutants. In either case, the situation could be corrected by sterilizing the waste, diluting it to reduce toxic effects and reinnoculating it with a fresh supply of microorganisms.

#### IV. CHEMICAL AND MECHANICAL DISPOSAL

#### A. Storage in Cans or Containers

Storage of feces in sealed cans is one of the methods proposed for disposal of human wastes in space flights of short to moderate duration. However, the carbon and oxygen contained in them is removed permanently from the life cycle, so this would not be a feasible method for use on interplanetary voyages. Moreover, carbon dioxide, noxious vapors, and sometimes methane, are generated in anaerobic fermentation of the wastes, which results in considerable pressures being developed within the cans. Leaks will occur unless the seams of the container are sealed very tightly. Therefore, it would seem advantageous to add a biostat to the wastes before closing the can. This should halt, or at least reduce, microbial action and therefore retard the development of high pressures. Besides avoiding leaks, this would reduce the size of the containers required, for in the absence of biostats considerable head space must be allowed for accumulation of the gases generated during fermentation.

Desiccation of the wastes to a moisture content of 8 percent or less would greatly retard microbial action, and so simplify the storage problem. Moreover, the recovered water could be returned to the life support system. Since recycling of oxygen and water are more critical in voyages of short duration than recycling of carbon, nitrogen and mineral nutrients, this could offer a distinct advantage on journeys of intermediate duration. However, equipment would be required for the desiccation of the wastes which might

increase the overall payload required. Even in voyages of relatively short duration, recovery of water from urine will be required. Therefore, purification of the water recovered from feces could probably be carried out along with this without imposing an additional penalty in payload.

Freezing of sealed containers has been proposed as a third method of preservation. This would not appear to be practical if artificial refrigeration must be used. However, it would look more practical if a storage compartment could be designed that would utilize the intense cold of outer space for preservation.

#### B. Zimmerman Wet Oxidation Process

The Zimmerman wet oxidation process consists of oxidizing wastes at a temperature of 500°F and a pressure of 1200 to 1800 psig. Air is fed into the reactor with a compressor to supply oxygen. If the wastes contain 4.4% or more solids, enough heat is generated during oxidation to make the reaction self-sustaining (ref. 3, 10). At higher solids contents, excess heat is generated which can be used for other purposes. However, the wastes must be preheated with an external source to initiate the reaction.

A flow diagram of a Zimmerman unit is shown in Figure 1. Sludge preheated to 180°F is pumped from storage tanks to a high pressure pump. Immediately after passing through the pump, air is introduced into the stream from a compressor. The sludge and air pass through a heat exchanger (No. 1) which is used only during the start-up period. The mixture then passes through a second heat exchanger (No. 2) which raises the temperature to approximately  $380^{\circ}$ F, and finally through a 3rd heat exchanger (No. 3) where the temperature

enters the bottom of the reactor, moves up through a coarsely baffled path in the central body of the reactor, and then spills over a port at the top of the inner shell into a jacket formed by the walls of the inner and outer shells. During the period of flow through the reactor, organic matter is degraded by wet oxidation. The reaction is complete when the mixture spills through the port. The end-products (at approximately 515°F) pass through the bottom of the reactor through the No. 3 and No. 2 heat exchangers where they serve to preheat the incoming wastes and air. The mixture then passes through a water cooler where the temperature is reduced to 150°F, and finally through a separator. In the separator, gases are vented to the atmosphere, and the liquid effluent containing the ash is withdrawn continuously from the bottom of the separator.

The composition of the effluent is shown in Tables 5 and 6. The effluent is characterized by high contents of ammonia and volatile acids. The BOD ranges from 5, 400 to 8, 400 mg/l which represents a reduction of about 60% from the BOD of the original waste. The settleable ash is between 10 and 12% of the volume after one hour settling time. The residual COD left in the ash from the effluent ranges from 1900 to 5000 mg/l and the BOD from 530 to 1350 mg/l. The volatile content of the dried ash ranges from 13 to 17% with an average of 15%.

The residue is sterile, and could probably be stored without bacterial decomposition. However, degradation of organic wastes is not sufficiently complete to make this unit attractive as a component of a self-sustaining life

support system. Undoubtedly, better results could be obtained by carrying out the reaction at higher temperatures and pressures and allowing for a higher detention time in the reactor.

Use of the Zimmerman process on a spacecraft would require a considerable scale-down which could lead to a number of difficulties. The smaller units now available are designed to handle 0.5 to 2 tons dry weight per day of sewage sludge (ref. 3). Undoubtedly a unit could be devised that would oxidize small amounts of feces if heat from an external source were supplied. However, it seems likely that a minimum of 2 kg per day (dry weight) of feces would be required for a self-sustaining operation. Otherwise losses of heat by radiation might be prohibitive. Also, the unit would require compressors, pumps and preheaters as well as the reactor unit itself, which would be unattractive from the standpoint of the increase in payload.

#### C. Dorr-Oliver Process

In the Dorr-Oliver process the waste sludge is concentrated to 35-40% total solids by passing it through a gravity thickener followed by centrifugation (ref. 2). The dewatered sludge is picked up by an air conveyer and fed into a fluidized bed reactor. The reactor contains a sand bed (60-80 mesh) which is fluidized by a stream of air. The solids burn rapidly in the bed at a temperature of 1500 to 1700 °F.

Stack gas from the reactor, which contains the inert ash, is passed through a water scrubber to entrain the solids, and cool the gases. This system cuts the bulk of the outgoing ash to 1.5% of the incoming solids.

The equipment appears to be more efficient than the Zimmerman process for the removal of organic matter. However, the use of a fluidized bed for burning the wastes does not appear attractive as a small scale operation.

#### D. Combustion Method

Human wastes could be degraded by removing water and organic volatiles by distillation, and incinerating the residue in the presence of oxygen. It would also be necessary to oxidize the organic volatiles since some of these would be odorous. Most of the organic material could be destroyed by ashing the residue at 850°C. The carbon dioxide could be stored as LiCO<sub>3</sub>, or used to support the growth of algae or higher plants in a photosynthetic gas exchanger. The ash could be used as a source of inorganic nutrients (Table 3). Studies have indicated that Chlorella pyrenoidosa will grow well on media containing 0.1 to 1.0 g/liter of waste ash fortified with sources of carbon and nitrogen plus a chelating agent. Concentrations of 5 g/l are toxic to algae.

Most of the energy input would be required to volatilize the water. Heat would be liberated on combustion of the organic residue. Some of this could be conserved by use of heat exchangers as in the Zimmerman wet oxidation process. It is possible that the energy requirement would not be a limiting factor if a nuclear reactor were used on board for the generation of electric power or for other reasons. Electrical energy may be at a premium on spaceships, but it is possible that heat energy may be more readily available as a by-product from power generators, propulsion units and perhaps directly from the sun.

More information is required on the fate of nitrogenous compounds during thermal combustion. If these are converted to elemental nitrogen, it would be necessary to include a nitrogen fixation unit in the life support system. This could be either a chemical unit (Haber process) or a microbiological unit (Rhizobium spp. Azotobacter spp., or Clostridium spp). If nitrogen oxides are formed, it would be possible to recover some of the nitrogen more directly through reactions such as:

$$NO + NO_2 \longrightarrow N_2O_3$$
  
 $N_2O_3 + H_2O \longrightarrow 2HNO_2$   
 $3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O$ 

#### V. MICROBIOLOGICAL DEGRADATION OF WASTES

Wastes can be degraded by microbiological oxidation or termentation as well as by chemical processes. During aerobic oxidation, some of the carbon and nitrogen are fixed in bacterial protoplasm, and oxygen is utilized. During anaerobic fermentation, some carbon and nitrogen are also fixed, but no oxygen is consumed. However, considerable amounts of methane are produced which must be oxidized subsequently, or disposed of in some other way. Hence, microbial oxidation, or fermentation followed by chemical oxidation, is no more sparing of oxygen than waste disposal by combustion, providing the reaction is carried to completion in both cases. Similarly, it is doubtful if microbiological oxidation when carried to completion would result in any great savings in energy compared to combustion if efficient heat exchangers were employed to conserve and utilize the energy liberated during the latter reaction. Moreover, some components of metabolic wastes such as cellulose, lignin, and keratin are degraded only slowly and by specialized microorganisms. This fact might eventually lead to the fixation of large amounts of carbon and nitrogen in semi-inert organic solids, unless precautionary measures were taken.

In summary, the microbiological oxidation of wastes is essentially a catabolic process in which there is a net release of energy, and simple molecules such as carbon dioxide and water are created from relatively complex organic compounds. By contrast, photosynthesis is essentially an anabolic process requiring a net energy input, during which complex organic molecules

are synthesized. The two processes are complementary, but they are not comparable either in the overall complexity of the tasks they perform, or the absolute need for them in a continuous life support system geared for long term operation. During biological oxidation, a rather simple task is performed in a complex way, while in photosynthesis (or alternately chemosynthesis) both the metabolic pathways and the end products are complex. It would be possible to by-pass microbiological oxidation altogether in the design of a life support system, but a photosynthetic system or its equivalent would be essential in any space craft which could not be provisioned for the duration of the projected trip.

Nevertheless, the microbiological disposal of wastes has several advantages that should be explored more fully. The first of these is purely mechanical. Microbiological oxidation can be carried out in aqueous suspension at ambient temperature or slightly above. Aeration of the suspension and a means of disposing of the end products is all that is required. The microorganisms do the rest. It is unnecessary to have combustion units which can be operated at high temperatures, compressors, and heat exchangers such as are required in combustion methods.

A second advantage is the fact that oxidation may not have to be carried to completion. This could allow for a considerable saving in the energy required to regenerate the wastes. Thus Moyer (ref. 17) has added feces to a combined culture of activated sludge and sewage algae. Undesirable odors are dissipated in a few hours, and part of the contents of the reactor can be harvested and fed to experimental animals. The wastes are

converted in part to carbon dioxide which is then reduced to foods by photosynthesis. However, it is probable that part of the wastes are incorporated directly into bacterial and algal protoplasm with a net saving of energy.

The possibility of conserving energy in a microbiological waste disposal unit can be illustrated more directly by using a system based on methane bacteria and algae as an example. Wastes would be fermented anaerobically to yield a mixture of 70% methane and 30% CO<sub>2</sub> as the primary gaseous products. The methane then would be used as a sole source of energy and carbon for the synthesis of protoplasm by Psuedomonas methanica. The carbon dioxide would be used in photosynthesis by the algae. However, only 30% as much light energy would be required for this step as would be the case if all the gas was oxidized to CO<sub>2</sub>, since part of the latent energy present in the wastes is conserved in the methane.

For the above reasons, it seems very unlikely that microbiological disposal of wastes will be a practical process unless used in combination with photosynthetic and chemosynthetic steps. Oxygen would be used in the process; the carbon dioxide evolved would have to be stored as LiCO<sub>3</sub> or bound to molecular sieve. Therefore, no net return of material or energy would be made to the life support system. It would take much less time and equipment to preserve the wastes with a biostat and store them.

#### A. Aerobic Waste Disposal

In an aerobic digestor, organic wastes are converted to carbon dioxide and water in the presence of oxygen. Concomitantly with this, some of the carbon and energy contained in the waste are used for the

synthesis of bacterial protoplasm. It is generally believed that the reduction of organic wastes is accomplished primarily by bacteria and that rapid removal depends upon their unrestricted reproduction or growth while using a minimum of the organic material for energy.

The aerobic process is relatively rapid, and the end products are nonacidic, relatively free from odor, and have low energy content. Under exceptionally favorable conditions, as much as 95 - 98% of the waste may be degraded.

The chief disadvantages of the aerobic process are: (a) a large volume of fluid is required, (b) a fairly large quantity of oxygen is required, and (c) carbon dioxide is liberated which must be fixed by molecular sieve, as lithium carbonate or utilized in photosynthesis by algae and higher plants.

Organic matter present in the wastes as suspended or colloidal solids is removed by coagulation, entrainment, absorption, or oxidation. The chief reactions which occur are as follows:

Organic matter oxidation -

$$C_x H_y O_2 + (x + 1/4y - 1/2z) O_2 \rightarrow x CO_2 + 1/2y H_2 O_2 - \Delta H$$
 (1)

Cell material synthesis -

$$n(C_xH_yO_2) + nNH_3 + n(x + 1/4y - 1/2z - 5)O_2$$
   
  $(C_5H_7NO_2)n + n(x - 5)CO_2 + 1/2n(y - 4)H_2O - \Delta H$  (2)

Cell material oxidation -

$$(C_5H_7NO_2)n + 5nO_2 \rightarrow 5nCO_2 + 2nH_2O + nNH_3 - \Delta H$$
 (3)

Equation (1) represents the conversion of organic materials to carbon dioxide and water with the liberation of energy. As noted previously, the synthesis of cell materials [Eq. (2)] occurs concomitantly with this. However, the cellular protoplasm itself is finally degraded to yield carbon dioxide, water, and ammonia. The synthesis of protoplasm by the microorganisms which carry out the degradation process is illustrated by Equation (2). Equation (3) represents the oxidation of cellular material. It has been calculated that the oxygen demand of the cellular material is 1.2 grams per gram of cell structure.

The most efficient aerobic method for the disposal of wastes is the activated sludge process. The activated sludge consists of flocks of gelatinous masses which contain bacteria and other microorganisms. Flocculation is caused primarily by the sticking together of particles by mucoids secreted by the bacteria. Formerly, it was believed that flocculation could be ascribed exclusively to the zoogloes-producing bacterium Zoogloes ramigers. However, since then a number of additional species have been isolated which are capable of forming flock similar to activated sludge (Table 7). These species include Bacillus cereus, Paracolobactrum aerogenoides, Nocarda astinomorpha, Florobacterium sp, Escherichia intermedium and others. Microscopic examination of the flock formed by the different bacteria indicates little difference in structure. Therefore, it is concluded that the formation of flocks is not due to any special zoogloeal-producing bacteria.

When wastes are added to activated sludge, two interdependent processes take place. These are clarification of the wastes, and oxidation. Both of these take place most rapidly when the waste and activated sludge are first mixed, providing, of course, sufficient oxygen is available. Clarification of the wastes (adsorption of the particulate matter) is substantially complete in the first 20 - 40 minutes. However, several days may be required for complete oxidation. A balance must be maintained between clarification and oxidation. If the oxidation rate is too slow, the sludge will lose much of its clarifying power. If, on the other hand, oxidation rate materially exceeds adsorption rate, much of the sludge will be destroyed by wet combustion so that the remaining flock becomes very small and compact. Either extreme reduces the effectiveness of the activated sludge process.

The general design of an activated sludge plant is shown in Figure 2.

The raw sewage wastes are run into primary settling tanks where the wastes are allowed to sediment for about one hour. The supernatant is transferred continuously to an aeration tank where air is bubbled through the suspension for an average detention time of about six hours. The aerated wastes are then pumped continuously into secondary settling tanks. Part of the sludge (about 25%) is returned to aeration tank for reprocessing. The effluent sludge is then thickened, after which it is ready for final disposal. In this process, the organic wastes are only partially degraded. The final sludge which is produced is relatively innocuous and can be used in a fertilizer. In self-regenerating life support systems such as would be used in space vehicles, it would be

necessary to complete the oxidation of the sludge by secondary chemical or microbiological processes in order to return the carbon tied up in this fraction to the life cycle.

The volatile portion of the wastes should contain a minimum of 7% nitrogen in order to maintain good settling and filtering characteristics.

Phosphorus requirements are variable. It is estimated that about one pound of phosphorus is required for the disposal of 90 - 170 pounds (BOD basis) of organic material. As noted previously, the oxygen demand is about 1.42 grams per gram of bacterial cell structure in the sludge. For optimum efficiency, oxygen must be supplied to the sludge at a rate equal to or greater than its rate of utilization. In the activated sludge process, this is usually accomplished by diffusion from aerators under turbulent conditions. The total oxygen requirement can be calculated from the quantity of organic matter removed and the concentration of sludge solids according to the following equation

$$O_2 = (1 - a) L_r + B s_0$$

where  $L_r$  is the amount of organic matter removed,  $s_0$  is the initial concentration of active sludge solids, and a and B are empirical constants which must be determined experimentally for each type of waste.

The rate of waste digestion observed in laboratory experiments increases about twofold for each 10°C temperature rise within physiological limits. However, this observation has not been confirmed in sewage plant operations. Very likely, this discrepancy results from other limiting factors which might occur in a large-scale waste disposal plant. Presumably, these would not be

important in the construction of a compact waste disposal unit for use in space vehicle. In general, the most effective temperature range for decomposition of domestic waste is from 9° to 30°C (ref. 19). If the predominating microorganisms are mesophilic, the reaction rate may be increased by raising the temperature of the unit to 37°C. With the use of thermophiles, the temperature of the unit could conceivably be raised to 65°C to obtain a higher digestion rate. Siu (ref. 20) lists one cellulolytic bacterium that has an optimum temperature of 65°C.

The sludge age is closely related to the time required for the processing of organic wastes. It is defined as the weight of the dry suspended solids in the aeration tanks divided by the rate of the dry suspended solids in the sewage. It can be calculated from the relation

Sludge Age (Days) = 
$$\frac{V \times s_a}{Q \times C}$$

where V is the volume of the aerator, s<sub>a</sub> is the average concentration of suspended solids in the aerator, Q is the sewage flow in mgd\* and C is the concentration of wastes added to the digestor. Under average operating conditions, the detention time of the wastes in the disposal system is estimated at 3 - 5 days. Very likely, this could be reduced by the development of strains of thermophilic bacteria capable of clarifying and oxidizing wastes at higher rates.

Domestic sewage is generally treated at an aerator concentration of 1,000 ppm of suspended solids. If the concentration is doubled to about \* Million gallons per day.

2,000 ppm, the activated sludge becomes voluminous and its settling properties deteriorate within about one week. Eventually, the process fails.

This results from the fact that a smaller quantity of bacterial culture exists than is able to accommodate the increased load and still retain normal biological and physical properties. However, it seems probable that bacterial species could be adapted to tolerate higher concentrations, and that this need not necessarily be a limiting factor in the design of a waste disposal unit for use on space craft. By using step aeration, it is possible to dispose of 50 pounds of BOD per day per thousand cubic feet of aerator capacity, based on yearly averages obtained from the operation of sewage disposal plants. In this process, the concentration of suspended solids in raw sewage can be reduced from an initial value of 133 ppm to 6 - 7 ppm. This represents an efficiency of about 95%. It is obvious that efficiencies of this order or greater would be required in a self-sustaining life support system such as would be used aboard a space vehicle.

## B. Anaerobic Fermentation

Waste digestion can be carried out in the absence of oxygen by obligate or facultative anaerobes. Direct microscopic examination of sludge or liquid from a digester reveals the presence of the usual types of bacteria, a few ciliates, colorless flagellates, and amoeba. Some of the bacteria which are present (Proteus vulgaris or Beggiatoa alba, for example) generate hydrogen sultide, which makes the process an odorous one. Pyrimidine production can also liberate nauseous odors. For this reason, an anaerobic digester would have to be isolated from the atmosphere of the spacecraft, and the effluent gases burned or disposed of in some other way.

Anaerobic digestion proceeds in two fairly well defined stages. These are liquefaction and gasification. The term liquefaction signifies the transformation of large solid particles of wastes into either a soluble or a finely dispersed state. This process proceeds by hydrolysis brought about by extracellular enzymes. These enzymes are excreted by microorganisms into the surrounding medium where they come into contact with the material they act upon. The microorganisms may also become attached to solid particles. When this occurs, the enzymes excreted by them do not diffuse into the surrounding medium and therefore do not become diluted. Consequently, some of the hydrolytic products may diffuse directly into the bacterial cell where they can be used for energy and growth rather than diffusing into the surrounding medium. This close association between the organisms, enzymes, and particles enhances digestion rate. Liquefaction is therefore a preliminary phase in which the wastes are prepared for final decomposition through conversion to gases. However, the liquefaction stage is not considered to be a secondary one, since without it gas production would not be possible. During the early stages of liquefaction, the BOD of the wastes increases, showing that they are converted to products which are more readily amenable to biochemical oxidation than the original materials.

Gasification consists of the conversion of the solubilized waste products to methane and carbon dioxide. The amount of methane produced may vary between 45 - 75% of the total volume of gas, depending on the substrate.

Methane production is essentially an intracellular procedure. It is carried out by methane bacteria such as Methanobacterium formicicum

or <u>Methanococcus mazei</u>. These microorganisms can decompose compounds such as formate, propionate, butyrate, acetate, methanol, etc., to form carbon dioxide and methane.

Methane organisms are strictly anaerobic. If the culture becomes exposed to oxygen even for a short time, an adverse effect is produced on the bacteria. The presence of bacteria which can produce highly reducing conditions is required for the successful growth of the methane organisms.

Methane organisms in pure culture can neither dispose of oxygen or create highly reducing conditions.

Methane organisms grow best in sediment and their development rates are slow. Therefore, large quantities of digested sludge are required for inoculation. Some organisms require an incubation period of one to ten weeks, depending on the size of inoculum, while others such as the coliforms may develop in 24 - 48 hours. Studies with pure or highly purified cultures of methane organisms have shown that they do not utilize substrates such as cellulose, glucose, proteins, amino acids and fats. Instead, they are restricted to the metabolism of simple compounds such as the lower fatty acids and alcohols. Thus, bacterial transformation of complex organic materials takes place in two stages. Complex organic molecules such as carbohydrates, polypeptides, amino acids, fatty acids, and alcohols are degraded to simple molecules by a variety of common bacteria. Methane bacteria then complete the transformation to carbon dioxide and methane.

The general equation for methane fermentation is given as follows:

 $4H_2A + CO_2 = 4A + CH_4 + 2H_2O$ 

where H<sub>2</sub>A represents any compound which can be activated by the methane bacteria to serve as a hydrogen donor for the reduction of carbon dioxide.

Gases other than methane are sometimes reported. These include hydrogen, small quantities of carbon monoxide and hydrogen sulfide.

Although present in relatively small amounts, the latter two gases are toxic and would have to be disposed of completely by catalytic oxidation if they were formed in an anaerobic waste disposal unit.

High-rate anaerobic sludge digestion is a process in which the contents of the primary digestion tank are completely mixed on a continuous or intermittent basis to maintain a uniform composition in all parts of the tank.

Wastes may be fed into the tank on an intermittent or continuous basis. However, in all cases, the mixed liquor is displaced from the tank rather than the supernatant liquor or sludge. Secondary units are often used as part of the digestion system. The principal requirement for maintaining high-rate digestion is to keep the entire contents of the tank uniformly mixed. This can be done by gas recirculation or mechanical stirring. The main drawback to the high-rate digestion procedure is that the mixture leaving the digestion unit for final disposal has essentially the same composition as the material left in the unit. This means that degradation will not be complete unless the unit is not fed for a prolonged period of time before harvesting the effluent. The situation is shown graphically by Figure 3.

From this, it can be seen that the amount of short residence solids contained in the effluent becomes a large part of the total when the detention time of the wastes in the digester is less than ten days. The data shown here were calculated on the basis of feeding the disposal unit once per day. If more frequent feedings are made, the situation becomes even worse. Therefore, a secondary disposal unit, probably operating on the aerobic principle, would be required to utilize the high-rate process.

Mesophilic sludge digestion can be carried out at temperatures up to 35°C. However, thermophiles can be developed by raising the temperature of the mixture slowly through a 90-day period. In this way, it is possible to increase the operating temperature to 55°C. Turnover rate is faster at thermophilic temperatures than at mesophilic temperatures. Under the former conditions, a loading of 0.7 pound of volatile solids per cubic feet per day could be used with a displacement time of less than 5 days. The average reduction in volatile solids is 83% and the BOD is reduced by 75%.

In general, anaerobic digesters can handle higher concentrations of solids than aerobic units. However, the reduction in BOD is not so great, and noxious end products such as hydrogen sulfide and pyrimidines may be formed. Moreover, many pathogenic bacteria thrive under anaerobic conditions. Therefore, the waste disposal unit might serve as a reservoir of parasites of this type.

However, the most undesirable feature of anaerobic digestion is the fact that methane is produced as one of the end products. This would render

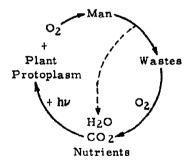
anaerobic digestion impractical unless a method was found for utilizing it.

Conceivably, it could be employed as a fuel for heating the digester, for incinerating the effluent, or for other purposes. Alternatively, it could be used as a source of energy and carbon for the chemosynthesis of protoplasm by Pseudomonas methanica. This possibility will be discussed in later sections.

#### VI. INTEGRATED PROCESSES

Microbiological degradation of human wastes would not offer any outstanding advantages over chemical oxidation or storage unless the process were coupled with photosynthetic or chemosynthetic systems. The bacteria would utilize some of the oxygen supply of the spacecraft in converting organic wastes to carbon dioxide and humins. Both of these products are innocuous, but they would take up storage space and at the same time withdraw carbon, oxygen and nitrogen from the life support cycle.

It would be much more efficient if a miniaturized, but complete life cycle could be established within the spacecraft environment, similar to that which has been developed through billions of years of terrestrial evolution. Of course, our own life cycle is not 100% efficient. The existence on earth of coal deposits, coral reefs, petroleum fields and peat beds testifies that microbial decomposition is not always capable of handling the metabolic debris produced by plants and animals. However, it is possible that a more efficient life support system could be developed for use aboard a spacecraft by careful selection of the various biological components on the basis of speed plus efficiency. The general scheme that would be used in such a life support system is illustrated below.



Organic wastes would be oxidized by bacteria to yield carbon dioxide, water and inorganic nitrogen. The wastes would also contain inorganic nutrients. The end products of bacterial oxidation then would be used by photosynthetic plants for the production of protoplasm. During the photosynthetic process, water would be split to yield molecular oxygen and a chemoreductant. The oxygen and the protoplasm produced by the plants would then be used by human or animal inhabitants of the spacecraft for respiration and food. Carbon dioxide and wastes would be produced again, and the cycle would be repeated. In an ideal system, carbon, nitrogen, inorganic minerals and the components of water would be reused continuously. The only external requirement would be an energy source which in this case would be light. Of course, such a unit would never operate with 100% efficiency. Humins and volatile components are produced by microbiological oxidation and would have to be degraded further by combustion or wet oxidation processes. However, it is probable that chemical treatment would have to be used for a relatively small proportion of the wastes.

Most of the systems proposed to date utilize aerobic bacteria for oxidation, and algae for photosynthesis. These may not always be the best choices as will be shown later; for example, anaerobic bacteria might be utilized for partial degradation of wastes with a substantial saving in energy. Moreover, considerable doubt exists whether man and higher animals can be sustained for prolonged periods on a diet which consists solely of algae. Foods prepared from them are not palatable, and some doubt exists as to whether the essential amino acids are present in ratios required for the support of

mammalian life. It seems likely that Hydrophytes, or other higher plants grown in tissue cultures or hydroponically, would have to be used to extend the algal diet. It is possible that animals might have to be introduced into the system at some point as a source of protein, although it is well known that the efficiency of caloric conversion in the transformation of plant products to meat is notoriously low.

These problems will undoubtedly have to be investigated in detail.

However, in the following sections, it will be assumed that algae will be the primary source of photosynthetic capability. This may not be a valid assumption, but it must be used temporarily until more reliable data on nutrition are available and advanced studies have been made using higher plants.

## A. Aerobic Process in Mixed Culture

It has often been suggested that the most efficient way to utilize wastes in the production of food would be to grow aerobic bacteria and algae in mixed culture. The bacteria would solubilize and oxidize the wastes, and the algae would use the products formed directly. Moyer (ref. 17) has investigated this process using mixed cultures of activated sludge and sewage algae in a 3:1 ratio. A container with a volume of 14 liters is loaded with 10 liters of mixed culture and fed with suspensions of homogenized human wastes. The detention time is 3 days and a holding volume of about 25 liters per man is required. Odors are absent within 2 hours after adding the wastes. Material was withdrawn from this unit and pelletized for use as food. Rats grown on it lost an average of 4 g the first week and 5.9 g in 21 days.

Although they were not able to maintain normal weight on this material, its nutritional value was evidently high enough to sustain life.

However, it:is not believed that products such as these could be used to sustain human life. There would be psychological objections to the presence of undecomposed waste particles in the food, and from the data presented it appears doubtful if the nutritional value would be high enough for use over prolonged periods.

## B. Symbiotic Growth of Cultures Separated by Membranes

## 1. General Description of Method

Growth of bacteria and algae in the same culture medium has several disadvantages. First among these is the fact that the material used for food would contain particles of undecomposed wastes. Moreover, the amount of light available for photosynthesis by algae will be reduced through absorption by colored materials present in the wastes. It has been found experimentally that algae grow best in such a medium when the waste is partly digested before innoculation. When the algae are added at the beginning of the process, growth is poor.

However, it is believed that a two-step process in which the bacteria and algae are grown entirely separately would be inefficient. Oxygen would have to be supplied to the waste unit from an external source throughout digestion, and the carbon dioxide evolved would have to be stored in some manner for subsequent use by the algae. Odorous gases and vapors would also have to be trapped and stored or otherwise disposed of. Thus, it would be more efficient if the algae and bacteria were able to grow symbiotically

this investigation which show that it is practical to grow algae and bacteria symbiotically in compartments separated by semi-permeable membranes such as cellophane or polyvinyl alcohol. Carbon dioxide produced by the bacteria is utilized by the algae for photosynthesis. In turn, the algae supply the bacteria with oxygen for respiration. Inorganic nutrients as well as simpler organic molecules can diffuse across the membrane to supply the algae with nutrients. The membrane serves to prevent bacterial cells and solid particles from contaminating the algal colony, thus eliminating problems due to poor light transmission through the algae culture and possible contamination by bacteria harmful to the algae.

A number of prototype models have been constructed to demonstrate the feasibility of this process. One of the later units is illustrated in Figure 4. This unit is constructed of Plexiglas and consists of a  $3 \times 26 \times 28$  cm chamber for holding the algae, and a compartment of similar size for holding the bacteria. The photosynthetic surface is  $420 \text{ cm}^2$ , and the surface available for aqueous diffusion is  $264 \text{ cm}^2$ . The surface available for the diffusion of gases through the membrane is  $165 \text{ cm}^2$ . Each compartment contains 1500 ml of suspension. In addition to liquid and gas inlet and outlet ports, this unit is equipped with thermometers and a cold water condenser in the air space above the algal suspension. Chamber A is a compartment which contains activated sludge bacteria and waste materials in fluidized form. Fresh wastes can be fed into A intermittently through inlet D. The two chambers are separated by a membrane B. H is an outlet

for harvesting the algae and water produced by the unit. To insure mixing of the atmosphere in both chambers, gas is circulated between the two units with a Sigmamotor pump. A condenser is supplied for condensing water vapor on the algal side.

In operation, wastes are fed into the system and through the action of the bacteria in A are converted to carbon dioxide, water and ammonia. The carbon dioxide produced in A is used by the algae in compartment C for photosynthesis. Urea and inorganic salts flow through the membrane B and supply the algae with nutrients. Every time waste is added, an equivalent amount of algal suspension is removed at H. This material consists almost entirely of algae and water. In a completely functioning unit, the water could be desalted by evaporation, electrodialysis or some equivalent process. It is hoped that a balance can ultimately be achieved in which the wastes are converted to useable materials by light energy with a minimal demand for supplementary nutrients. It is felt that contamination of the algae and toxic interactions between the two populations can be minimized with the type of equipment described, and still obtain the advantages of a combined unit over two separate units.

Experiments have been carried out over periods of 21 days in which algae were grown on the oxidation products liberated by activated sludge bacteria. However, this was not a completely functioning unit. The chief difficulty encountered was in getting a sufficient amount of water to pass through the semi-permeable membrane to allow the unit to be fed and

harvested on a continuing basis. It is believed, however, that this objective could be accomplished in future designs by use of highly efficient condensers in the algal compartment. Liquids will not pass through membranes such as cellulose readily except under high pressures. However, diffusion of water vapor in the gas phase through cellophane is about 75% as efficient as diffusion through free air. Therefore, if a way could be found to reduce the relative humidity in the algal compartment through condensation of water vapor, it would appear that water transfer could be carried out efficiently. This aspect of the problem was not solved during the current study. However, it is believed that further work on this point could lead to the construction of a unit which would demonstrate the overall feasibility of the process.

From a theoretical standpoint, there appears to be no valid reason why it would not operate satisfactorily. In experiments carried out so far, good growth of algae was obtained over a period of 20 days accompanied by satisfactory decomposition of the waste. During this period, the composition of the air within the chamber remained constant at 20% oxygen and 80% nitrogen even though it was sealed from the laboratory atmosphere. This indicates that wastes were being degraded by bacteria and resynthesis was taking place at approximately equivalent rates.

More work will be required on the selection of membranes. In earlier models, two membranes were used which were separated from one another by a barrier consisting of 1/2" of distilled water. This expedient was used as a safety factor in case one of the membranes failed. During these experiments, the membranes remained intact for a period of 20 days. However, when the

same membrane (cellophane) was used in the apparatus shown in Figure 4, holes appeared in it within a period of 4-5 days. In these experiments, the algae and fungi were both in direct contact with the same membrane. It is not known whether deterioration of cellulose was caused by symbiotic action. However, it is noteworthy that failure of the membrane did not occur in experiments where two membranes separated by a barrier of distilled water were used. Better results were obtained in the single membrane experiments by replacing the cellophane with polyvinyl alcohol films hardened by heat treatment. Hardening is necessary to decrease solubility in water. If the film is insufficiently hardened, it will absorb considerable quantities of water and sag. This renders measurement of water transfer rates difficult.

In order to evaluate the membrane method more fully, it will be necessary to evolve a more efficient method of water transfer, and to improve the physical stability of the membranes. However, it is felt that these are purely mechanical problems that could be solved with relatively little effort.

Of course, it is realized that this design may be far too simple to serve as the prototype of an operative unit. In the first place, odorous gases might be produced which would contaminate the algal colony. Moreover, it is possible that toxins might be built up which would diffuse across the membrane and contaminate the algal culture. To avoid these potential difficulties, a more complex system could be used. One such possibility is shown in Figure 5. Two units would be provided for the disposal of solid wastes (S<sub>1</sub> and S<sub>2</sub>). This would permit one of them to be shut down at any time to remove humins. These units would be connected through a semi-permeable

membrane to a unit designed for the disposal of diffusable waste (L). This unit would be inoculated with activated sludge or possibly with selected species of bacteria. The algae (or other photosynthetic plants) would be contained in compartments  $A_1$  and  $A_2$ . The bacteria in the unit L would be expected to destroy undesirable materials diffusing from the primary units  $S_1$  and  $S_2$ , before they could enter the algal compartments. Gas exchange would have to be regulated in such a way that noxious material did not accumulate in the algal compartments, although, in the experiments conducted, this did not appear to be a serious problem.

## 2. Experimental Procedures

# a. Preparation of Cultures for 2-Chamber Digestion Process

In the foregoing experiments, synthetic wastes were prepared from 15 grams of dehydrated cooked meat, 300 ml of distilled water and one gram of papain. This mixture was homogenized in a Waring blender for several minutes and then allowed to digest for two hours. Following the digestion, 60 ml of urine was added and the suspension was diluted with distilled water to a volume of 3 liters. This mixture had a pH of 5.9.

Three liters of Kratz (ref. 14) modified algal medium containing no nitrogen was prepared. The pH of this mixture was 7.4. The two solutions were added to opposite sides of the membrane of the double chambered Plexiglas 44 digester unit. The synthetic waste was inoculated with 10 ml of freshly settled sewage and the algal medium was inoculated with a pure culture of Chlorella. In some of the latter experiments, freshly settled sewage was used in the waste side of the unit. The pH of this sewage was 7.1.

The medium selected for growing algae was one developed by Kratz and Meyers (ref. 14), usually referred to as Kratz's medium. It has the following composition:

Ingredient	Amount (g)
MgSO4.7H2O	0.25
K2HPO4	1.00
Ca(NO3)2.4 H2O	0.025
KNO <sub>3</sub>	1.00
EDTA	0.05
Fe(SO <sub>4</sub> ) <sub>3</sub>	0.004
Micronutrient Solution	1 ml

The micronutrient solution is prepared by making a mixture of the following compounds up to a volume of 1 liter with distilled water.

Ingredient	Amount (g)
H <sub>3</sub> BO <sub>3</sub>	2.86
MnCl <sub>2</sub> . 4 H <sub>2</sub> O	1.81
ZnSO4.7 H2O	0.222
MoO3 (85%)	0.0177
CuSO4. 5 H2O	0.079

# b. Analysis of the Digestion Atmosphere

The air samples withdrawn from the combined waste disposalphotosynthetic units were analyzed by gas-solid chromatography according to
standard procedures (ref. 4). Carbon dioxide, nitrogen and oxygen can be

analyzed in air samples by passing the sample through columns packed with molecular sieve and silica gel. Two packings are generally used, which can be arranged in parallel or in series. If this equipment is not available, duplicate air samples can be analyzed on first one packing and then the other. The silica gel column separates carbon dioxide from air but does not resolve oxygen and nitrogen. However, oxygen and nitrogen can be separated from one another on molecular sieve. This latter packing binds carbon dioxide irreversibly at room temperatures which is the reason why two columns must generally be used for this analysis. A typical elution pattern is shown in Figure 6.

During the two-chamber experiments, the composition of the atmosphere in either chamber did not vary appreciably from normal. (Table 8)

# c. Metabolism of Urine Constituents

Since urine may be one of the substances which must be treated in a waste disposal unit, it became of interest to establish the identities of the growth-limiting chemicals contained in it. In addition, information was required on the utilization of nitrogenous compounds by algae. To answer this first question, an experiment was set up in which uricacid and creatine were added to complete Kratz medium in various concentrations (Table 9). Solutions containing these compounds at the concentrations indicated were prepared and inoculated. Absorbance readings were made daily at 456 mm to determine relative growth rates. It is known that absorbance is not always directly proportional to cell concentration. However, in this experiment it was shown that Beer's law was followed in a dilution series. The results are tabulated

in terms of percent of control (complete Kratz medium). It can be seen that urea at a concentration of 0.5% did not have a pronounced effect on growth rate. Growth appeared to occur more rapidly than in the control between 4-10 days but thereafter was the same. In all cases, uric acid at concentrations ranging from 0.012 to 0.096% appeared to enhance growth. This effect was more prenounced at the higher concentrations of uric acid. The results obtained on creatinine were generally higher than on the controls, but in most cases the difference was not highly significant.

Measurements were made next on Kratz medium in which the nitrogen source was left out and replaced by an equivalent amount of nitrogen contained in urea, uric acid and creatinine (Table 10). Growth on the medium deficient in nitrogen fell off rapidly compared to the control (complete Kratz medium), and at the end of 24 days the absorbance was only 15% as high. By contrast, the rate of growth of the algae on an equivalent amount of urea surpassed that of the control for the first two weeks by a fairly substantial amount. However, by the end of 24 days, growth was substantially equivalent to the control. Uric acid appears to be a more effective nitrogen source than urea. Growth rates obtained on it were significantly higher throughout the entire course of the experiment, and at the end of 24 days the population density (as measured by absorbance) was over twice that of the control. However, creatinine did not appear to be an efficient nitrogen source in this particular experiment. Growth rate dropped to about half that of the control in 14 days and was only one-quarter of that obtained on the control at the end of the experiment. However, this was almost twice the

growth obtained on the medium which was completely nitrogen deficient.

The results obtained on creatinine do not agree with earlier work in which
a stimulatory effect similar to that obtained with urea was found.

It appears that both urea and uric acid can be utilized directly by algae as nitrogen sources. Creatinine is not used as efficiently, although it apparently will support growth to some extent. These observations are reassuring in that the diffusion of uric acid and creatinine across the semi-permeable membrane separating the algal and bacterial cultures is not likely to be a serious problem. Evidently these compounds can be used by algae as a source of nitrogen, unless growth is inhibited by high concentrations of them.

# d. Metabolism of Proteinaceous Wastes

In municipal sewage plants, soluble wastes are flocculated by activated sludge and digestion is continued until the sludge becomes stabilized. The material can then be dumped or used as a soil conditioner. However, in a space vehicle it would be desirable to recycle all of the carbon. Therefore, storing or jettisoning stabilized sludge would probably be undesirable. Anaerobic digestion of stabilized sludge is very slow. Moreover, noxious gases are produced which might create an atmospheric pollution problem. However, aerobic digestion of the solids may provide a partial answer to the problem. It has been found that a 50-60% reduction of solids can be obtained in a 4-6 day retention time.

In order to demonstrate the maximum reduction in BOD which could be obtained by aerobic digestion, a series of experiments was

conducted in which synthetic wastes derived from the hydrolysis of meat were digested by sewage bacteria. Destruction of organic matter as measured by five-day BOD values proceeded slowly at first, but gained momentum as the bacterial population increased (Figure 7). At an initial concentration of 5, 900 mg per liter (BOD), the time required for decomposition for one-half of the waste was nine days. At the end of 22 days, the BOD was reduced to 34 mg per liter so that wastes were reduced at an average of 267 mg per liter daily. However, at the end of this time, some solid material remained. At an initial BOD of 3950 the half time was eight days and at 1515 it was three days, The residual BOD decreased in rough proportion to the initial loading. Similar experiments were carried out using an acclimated microbial culture (Figure 8), but digestion appeared to be somewhat slower. These results indicate that the average detention time for the proteinaceous components of waste would be on the order of 8-10 days using a batch process in which the wastes are treated with fresh inoculum. However, it would be much more desirable to use a system in which waste could be treated continuously. In this system, more rapid turnover could be obtained by having a high bacterial population available at the onset of each loading. This would eliminate the lag.

## C. Anaerobic-Aerobic Digestion Methods

Anaerobic digestion is not considered to be a feasible process for waste disposal unless a use can be found for the methane which is produced.

One possibility is to use this gas as a source of heat for carrying out the digestion thermophilically, or for incinerating undigestible residues. However,

this would require the utilization of atmospheric oxygen and at the same time waste energy. It might be more economical to use bacteria to oxidize the methane and conserve the energy in the form of protoplasm.

This could be accomplished by a bacterium known as Pseudomonas methanica. This organism is an aerobe which can utilize methane as a sole source of carbon and energy. As a matter of fact, it has an obligate requirement for methane and will not grow on ordinary substrates such as glucose. It can utilize ethane and propane in small amounts but not as a sole source. Evidently the chemosynthetic activities of this organism are built around one carbon metabolism. The bacteria can use ethane and propane as an energy source, but apparently cannot incorporate them into metabolic intermediates.

This unique requirement for methane suggests a way in which these bacteria could be used to reduce the amount of energy required for photosynthesis. Anaerobic fermentation of organic wastes yields a gas which contains 40-75% methane, the remainder being primarily CO<sub>2</sub>. The carbon in CO<sub>2</sub> is in a completely oxidized state so energy must be supplied for its incorporation into protoplasm by algae or other photosynthetic plants. However, carbon in methane is in a completely reduced state and will yield energy on oxidation. Therefore, by using Pseudomonas methanica to oxidize the methane, and algae to reduce the carbon dioxide, it might be possible to lower the energy requirement for the disposal of solid human wastes by as much as 40-70%, depending upon the methane yield on anaerobic fermentation.

The apparatus in which this has been accomplished on a laboratory scale is shown in Figure 9. A is an anaerobic digestor which contains liquifing and gasifying bacteria. The contents of this unit are inoculated heavily with anaerobic sludge, and the air above the liquid is displaced with nitrogen. On fermentation, this unit yields a mixture of methane and carbon dioxide. The gases pass out of this unit under pressure through a one way valve. They are then recirculated continuously by the air pump P through a suspension inoculated with algae (C) and a culture inoculated with methane bacteria (B). The CO2 and methane produced in the anaerobic step are thus circulated continuously through the bacterial and algal cultures. Carbon dioxide is fixed by the algae through photosynthesis, while the methane is used by Pseudomonas methanica for chemosynthesis. These activities are complementary. The methane bacteria require oxygen for the fixation of carbon and the production of high energy compounds, and in this process some of the methane carbon is liberated as carbon dioxide. The algae can use this carbon dioxide plus the carbon dioxide derived from the anaerobic fermentation process in photosynthesis. In the photosynthetic process, oxygen is formed which is subsequently used by the methane bacteria. Hence, this system represents an arrangement by which anaerobic fermentation, photosynthesis and chemosynthesis are carried out concurrently with a net conservation of energy.

Quantitative studies have yet to be made on this process. Undoubtedly it would be necessary to develop bacteria with high growth rates in order to make the process successful. Moreover, little is known concerning the chemistry

of methane bacteria with regard to ratios of essential amino acids, etc. In the future, such studies should be made in order to determine if <u>Pseudomonas</u> methanica can serve as a food source, either directly or through the agency of intermediate microorganisms such as the edible fungi.

#### VII. CONCLUSIONS

A study has been made of various methods of waste disposal that might be used on space voyages of short, intermediate, and long duration. In general, the processes and equipment have not been developed to the point where the engineering aspects of the various methods can be compared on a quantitative basis. However, the following generalizations can be made:

- (1) On voyages up to 1000 days, degradation by chemical or micro-biological methods would appear to be impractical. The best solution might be dehydration, treatment with a biostat, and storage in hermetically sealed containers.
- (2) On voyages in the neighborhood of 1,000 days or over, recycling of the wastes would probably be advantageous. Regeneration would probably be carried out through photosynthesis, but oxidation could be carried out by chemical or microbiological methods.
- (3) There would be no advantage to oxidizing wastes by any method unless the end products were used in photosynthesis. Concomitantly, there would be no advantage in including a photosynthetic step unless the plant protoplasm was used as food.

  Recovery of oxygen from carbon dioxide can probably be accomplished more efficiently by chemical methods.

- (4) Chemical oxidation could be carried out with a net release of energy. However, the amount of waste produced by a crew of 1 to 6 would be too small to make a scale-down of municipal sewage disposal methods (Zimmerman and Dorr-Oliver) practical. More efficient units could be designed for continuous wet or dry combustion on a small scale. However, it is estimated that the feed would have to be of the order of 5 kg/day to design a unit that would be self-sustaining energetically. The most practical method of chemical oxidation on a small scale would be through the use of by-product heat derived from other onboard equipment such as nuclear powered turbine generators, methane fuel cells, nuclear and ion propulsion units, etc.
- (5) Aerobic digestion by the activated sludge method is rapid, but the concentrations of BOD that can be handled are low compared to the anaerobic process. However, breakdown of the organic matter is more complete, the end products are not noxious, and pathogens do not thrive in this environment. Therefore, it is probable that an aerobic step would be included in any integrated microbiological waste disposal process.
- (6) Aerobic disposal of wastes is possible through the joint action of activated sludge bacteria and sewage algae in mixed culture. However, this process does not appear to be attractive because of the probable presence of identifiable particles of waste in the harvested material and its questionable nutritional value.

- (7) An alternate symbiotic process is proposed in which the activated sludge bacteria and the photosynthetic organisms (algae and/or higher plants) are separated by semipermeable membranes. This prevents contamination of the food source by waste particles and bacteria but, at the same time, allows for free transport of respiratory and photosynthetic gases, inorganic nutrients, and soluble foods. Preliminary laboratory investigations indicate that this is one of the more promising techniques for the microbiological disposal of wastes. However, further engineering and design studies would be required to develop a fully functioning unit.
- (8) Anaerobic digestion is slow, but comparatively high concentrations of solids can be tolerated. However, the residual BOD is high, pathogens thrive in this environment, and noxious products such as hydrogen sulfide, carbon monoxide, and pyrimidines are produced. The main problem is that the digester gas consists mostly of methane and carbon dioxide. The carbon dioxide could be used in photosynthesis, while the methane could be burned to heat the digester to thermophilic temperatures, or to combust the residual BOD in the effluent. However, this would consume additional on-board oxygen supplies and require elaborate equipment. Preliminary experiments have been carried out in which algae and methane bacteria were grown symbiotically on a base feed of carbon dioxide and

methane derived from the anaerobic fermentation of wastes.

Additional work on the relative growth rates of bacteria and algae and other kinetic factors will be required before the practicability of the method can be assessed.

- (9) The chief advantage of anaerobic digestion might be as a means of conserving chemosynthetic energy. Theoretically, as much as 50% of the latent energy contained in wastes might be utilized by combining the activities of methane producing bacteria, methane utilizing bacteria, and green plants into a unified life support system.
- (10) A general discussion is included on the possible effects of weightlessness, radiation, and other space hazards on the operation of biological waste disposal units. This discussion cannot be quantitative, since methods and design features have not been stabilized, and insufficient basic information is available on the nature of the hazards involved.

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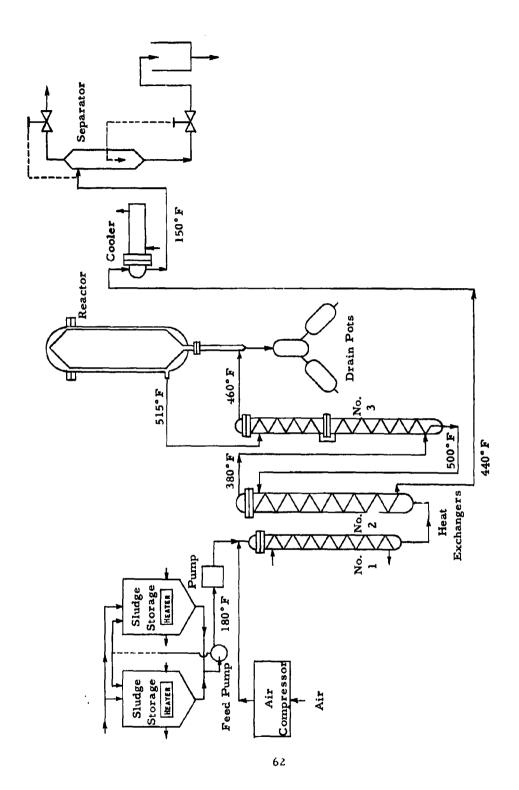


FIGURE 1. SCHEMATIC FLOW DIAGRAM OF A ZIMMERMAN WET OXIDATION UNIT (REF. 10)

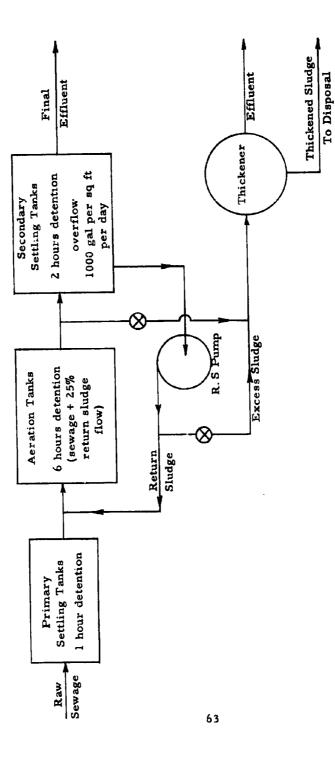


FIGURE 2. FLOW DIAGRAM OF A CONVENTIONAL ACTIVATED SLUDGE PROCESS (REF. 23)

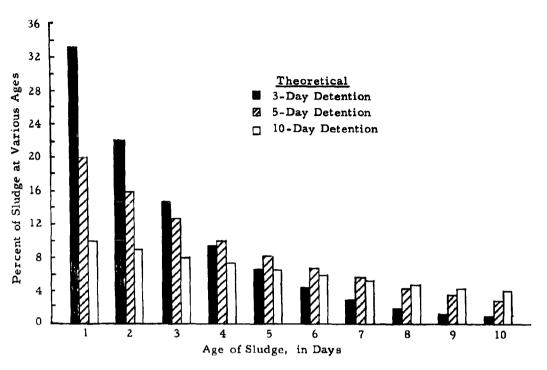


FIGURE 3. RELATIONSHIP BETWEEN DETENTION TIME AND AGE OF SLUDGE FROM HIGH RATE UNITS FED ONCE DAILY. (REF. 19)

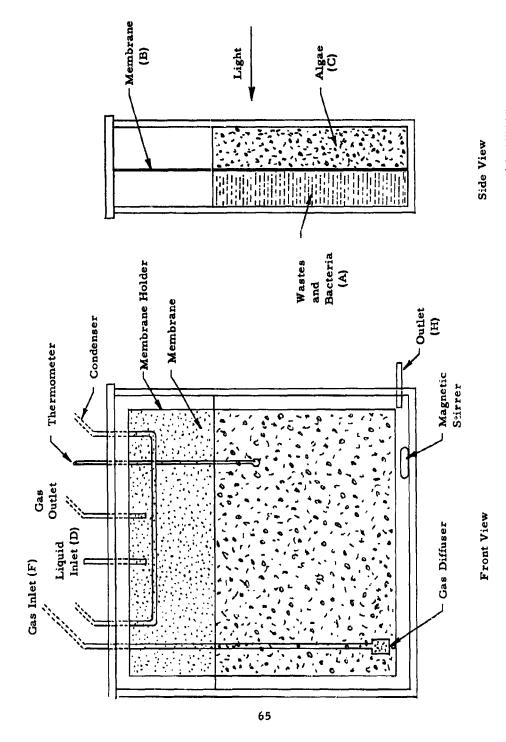


FIGURE 4. PROTOTYPE APPARATUS DEVELOPED FOR WASTE DISPOSAL BY BACTERIAL AND ALGAL CULTURES SEPARATED BY SEMIPERMEABLE MEMBRANES

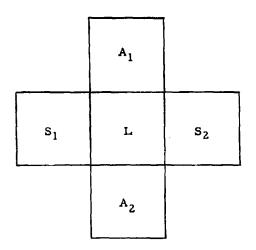


FIGURE 5. PROVISIONAL DESIGN OF A 5-COMPARTMENT UNIT FOR SYMBIOTIC WASTE DIGESTION IN WHICH THE SOLID WASTE COMPARTMENTS ARE SEPARATED FROM THE ALGAL COMPARTMENTS BY A LIQUID WASTE DIGESTER

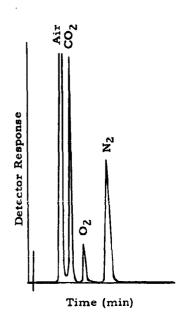


FIGURE 6. SEPARATION OF OXYGEN, NITROGEN, AND CARBON DIOXIDE BY GAS-SOLID CHROMATOGRAPHY

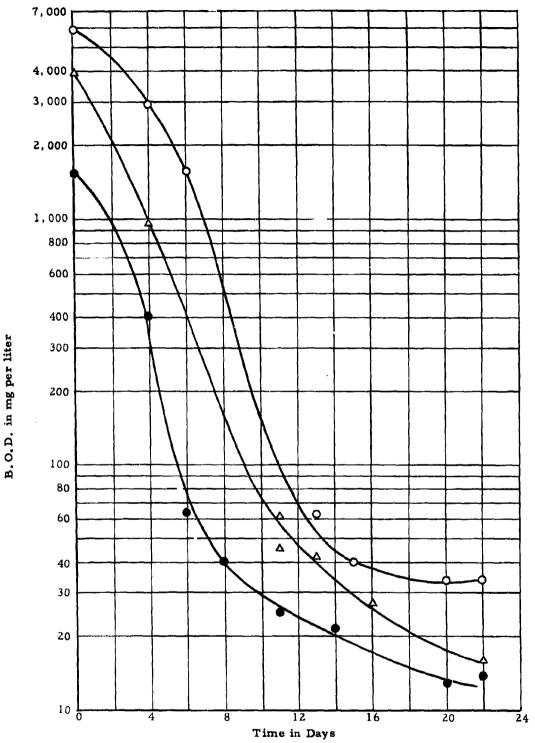


FIGURE 7. RATE OF DIGESTION OF SYNTHETIC WASTES AT 3 CONCENTRATIONS BY ORGANISMS IN SEWAGE 68

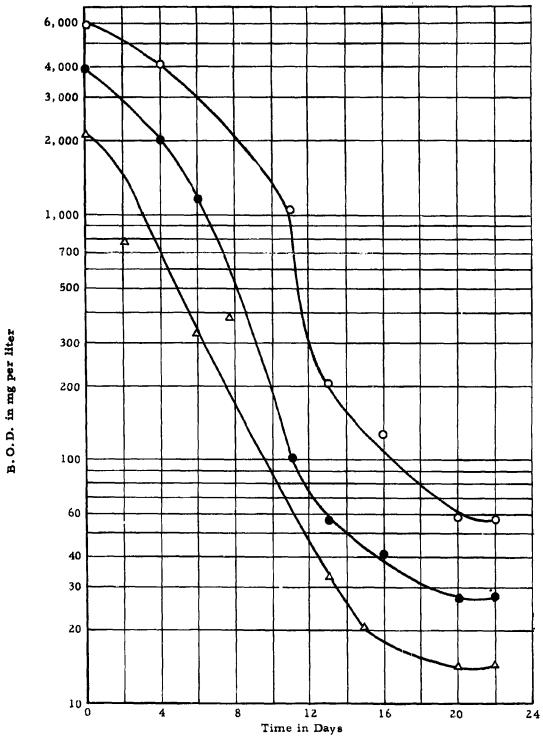


FIGURE 8. RATE OF DIGESTION OF SYNTHETIC WASTES AT 3 CONCENTRATIONS BY AN ACCLIMATED MICROBIAL CULTURE  $69\,$ 

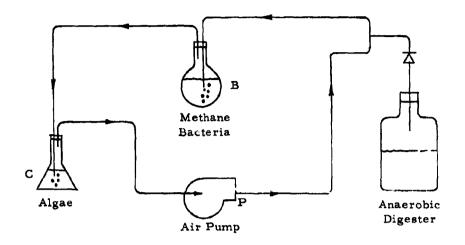


FIGURE 9. SCHEMATIC DIAGRAM OF A PROPOSED WASTE DISPOSAL UNIT EMPLOYING ANAEROBIC DIGESTION, CHEMOSYNTHESIS BY METHANE BACTERIA, AND PHOTOSYNTHESIS

TABLE 1. PROXIMATE COMPOSITION OF FECES (REF. 6)

Component	Weight (gm)	Percent of Total
Bulk	150	
Water	99	66.0
Dry Matter	27	17.8
Fat	4.7	3.0
Protein	?	?
Nitrogen	1.5	1.0
Carbohydrate	?	?
Minerals	2. 1	1.4
Sodium	0.12	
Potassium	0.47	
Calcium	0.64	
Magnesium	0. 20	
Chloride	0.09	
Phosphorus	0.51	
Sulfur	0.13	
Trace Elements: (ref. 2)		
Copper		
Iron		
Lead		
Manganese		
Nickel		
Zinc		
Arsenic		
Vitamins (ref. 15)	0.015	0.01
Bile Pigments	0.15	0.1

## TABLE 2. MINERALS AND TRACE ELEMENTS EXCRETED IN HUMAN FECES (REF. 1)

Substance	Amount: Mean Quantity and Range (µg/kg of body wt per day)
Aluminum	0.6
Arsenic	33(1-116)
Calcium	7490 (5000-10,000)
Chloride	(210-500)
Cobalt	0.007 (0.002-0.02)
Copper	27 (23-37)
Iron	120 (65-208)
Lead	4. 2 (2.2-19.8)
Magnesium	2500 (1510-3185)
Manganese	(18-120)
Mercury	0.14
Nickel	(1, 2-2, 5)
Phosphorus	9860 (7100-20,000)
Potassium	6,700
Silver	0.8
Sodium	1,700
Sulfur, total	2000
Tin	(170-450)
Zinc	100 (58-144)

TABLE 3. COMPOSITION OF URINE IN TERMS OF GRAMS PER 24 HOUR SAMPLE (REF. 24)

	Ordinary Diet	High Protein Diet	Very Low Protein Diet
Volume, ml	1250	1550	950
Total nitrogen	13. 20	23.28	4.20
Urea	24.30	43.80	6. 20
Urea nitrogen	11.40	20.40	2. 90
Ammonia	0.50	1.00	0.20
Arnmonia nitrogen	0.40	0.80	0.17
Creatinine	1.64	1.72	1.60
Creatinine nitrogen	0.61	0.64	0,60
Uric acid	0.60	06.0	0.30
Uric acid nitrogen	0.20	0.30	0.10
Total amino acids	1.5, less than half free		
Amino acid nitrogen	0.2		
Hippuric acid	0.6		
Indican	0.01		
Allantoin	0.015		

TABLE 3. COMPOSITION OF URINE IN TERMS OF CRAMS PER 24 HOUR SAMPLE (REF. 24) (Cont'd)

	Ordinary Diet	High Protein Diet	Very Low Protein Diet
Creatine	0.0-0.06		
Undetermined nitrogen	0.60	1.10	0.50
Glucose	0.10		
Nonglucose reducing substances calculated as glucose	1.0		
Phenols	0.20		
Citric acid	0.30		
Ascorbic acid	0.025		
Oxalic acid (oxalates)	0.015		
Acetone bodies	0.01		
Thiamine	0.0001		
Total sulfur	1.0		
Inorganic sulfates	0.80		
Ethereal sulfates	0.08		
Neutral sulfur	0.12		

TABLE 3. COMPOSITION OF URINE IN TERMS OF GRAMS PER 24 HOUR SAMPLE (REF. 24) (Cont'd)

	Ordinary Diet	High Protein Diet	Very Low Protein Diet
Chlorine as chloride	7.0		
Phosphorus as phosphate	1.0		
CO <sub>2</sub> as HCO <sub>3</sub> + H <sub>2</sub> CO <sub>3</sub>	Varies with urinary pH		
Sodium	5.0		
Potassium	2.5		
Calcium .	0.2		
Magnesium	0.15		
Copper	0.00004		·
Iron	0.00003		
Iodine	0.00005		
Water	90-95 percent		

## TABLE 4. VOLATILE COMPOUNDS IDENTIFIED OR TENTATIVELY IDENTIFIED IN THE ATMOSPHERE OF A SIMULATED SPACE CABIN (REF. 15)

Compounds Identified in Cabin Air by GLC	Max. Conc., μg/liter
Acetaldehyde <sup>2</sup> Acetone <sup>1</sup> Methyl Alcohol <sup>1</sup> Ethyl Alcohol <sup>1</sup> Freon-12 <sup>1</sup> Freon-11 <sup>1</sup> Methyl Chloride <sup>1</sup> Freon-22 <sup>1</sup> Dimethyl Sulfide <sup>2</sup>	0.53 2.05 0.58 16.6 185.0 47.0 2.3 0.6 0.012
Compounds Suspected in Cabin Air by GLC  Freon-1143	0.08
Isoprene <sup>2</sup> Acetylene <sup>4</sup>	0.08 0.18
Ethylene Oxide <sup>4</sup> Carbon Tetrachloride <sup>4</sup> Methyl Ethyl Ketone Benzene <sup>4</sup> Toluene <sup>4</sup> Propionaldehyde Isobutyraldehyde Methyl Isobutyl Ketone Methyl Propyl Ketone	0. 2 0. 035 0. 3 0. 2 0. 13 0. 011 0. 006 0. 31 0. 40
Unknown A Unknown B	0.57 5.3

#### TABLE 4. VOLATILE COMPOUNDS IDENTIFIED OR TENTATIVELY IDENTIFIED IN THE ATMOSPHERE OF A SIMULATED SPACE CABIN (REF. 15) (Cont'd)

Materials Detected by Chemical Tests	Max. Conc., ug/liter
Sulfate ion	$1.95 \text{ mg/m}^3$
Chloride ion	$1.80 \text{ mg/m}^3$
Sulfur dioxide	0.056 ppm
Chlorine	0.03 ppm
Ammonia	2. 64 ppm
Ozone	0.013 ppm

#### Identification

- 1. Infrared spectra of collected fraction
- 2. Odor + retention time
- 3. Halide detector + retention time
- 4. Retention times on two different columns

TABLE 5. CHEMICAL CHARACTERISTICS OF REACTOR EFFLUENT OBTAINED IN THE ZIMMERMAN WET OXIDATION PROCESS (REF. 10)

	П	₩ 00 00 00 00 00 00 00 00 00 00 00 00 00	Left in	Ach	mgi.	ò		1,900	3 400		5,000
	ffluent As	BOD#	Left in	Ash	(mg)		c L				1,350
	i i	Volume	Settled	in 1 hr	(m1/t)		Ç	70	105	) ·	116
ıge			Effluent	COD	(mg/t) (mg/t)		4 896 9 200	0, 200	9,800		11,600
each Ran			Settled	BOD	(mg/1)		4 800	7 C C	6, 410	:	7, 110
Average of Each				COD	(mg/1)		10, 200		13, 400	16 100	10, 600
	Reactor Effluent	Volatile Acids as	:	BOD	(mg/1)		5, 420	000	, 030	0 440	0, 400
			Acids as	Acetic	(mg/1)		3,200	7 700	7, 400	3 000	2,700
	Kea			org. N	(mg/1)		368	425	7	278	
			;	NH3-N	(mg/1)		1,370	1 625		I. 640	
	Volatile Calida	Concentration	Beneration	Vange	(percent)		2.00 - 2.99	3.00 - 3.99	00 7	4.00 - 4.99	•

\*BOD and COD left in ash settled from one liter of reactor effluent (by difference, reactor effluent-settled effluent).

TABLE 6. TREATMENT OF ZIMMERMAN PROCESS EFFLUENT BY ACTIVATED SLUDGE IN A LABORATORY EXPERIMENT (REF. 10)

	BOD	Q		Mixed Liq	nor Solids			
Aeration	Sewage		Beginning	Beginning of Period End of Period	End o	f Period	Hd	5.74
Period (hr)	Mixture (mg/t)	Effluent (mg/t)	SS (mg/1)	(percent)	(mg/1)	(percent)	of Period	Period
4, 5 (1st period)	311	45	3, 490	29.2	3,620	26.5	8.2	<b>8.4</b>
4. 5 (2nd period)	308	22	3, 540	27.2	3,650	23.8	<sub>د</sub> م	8. 12.
4. 5 (3rd period)	355	21	3,250	26.1	3, 470	24.5	& 	8.6
8 (4th period)	348	6	2,900	25.9	2,900	21.9	. 3 . 3	9.6

The wet-oxidation effluent was proportioned to the raw sewage on the basis of 600,000 gal of 6 percent sludge per day (150 tons dry) to 800 mgd sewage plus 50 percent return sludge. Effluent-sewage mixtures were aerated for time shown, then allowed to settle 1/2 hr. Supernatant was siphoned off and replaced with fresh amount of effluent-sewage mixture. None of the sludge was removed.

## TABLE 7. BACTERIA WHICH CAN FORM FLOC SIMILAR TO ACTIVATED SLUDGE (REF. 16)

### Active During First Phase

	Organism	Habitat
1.	Zoogloea ramigera	activated sludge
2.	Bacillus cereus	soil
3.	Escherichia intermedium	soil
4.	Paracolobactrum aerogenoides	soil
5.	Nocardia actinomorpha	soil
6.	Flavobacterium sp.	water

### Active During Second Phase

	Organism	Habitat
1.	Escherichia coli	intestinal canal
2.	Escherichia freundii	soil
3.	Pseudomonas perlurida	soil
4.	Pseudomonas ovalis	soil
5.	Pseudomonas segnis	trickling filter
6.	Pseudomonas solaniolens	soil
7.	Pseudomonas fragi	soil
8.	Alcaligenes faecalis	intestinal canal
9.	Alcaligenes metalcaligenes	intestinal canal
10.	Bacillus cereus	soil
11.	Bacillus lentus	soil
12.	Zoogloea ramigera	activated sludge

TABLE 8. PERCENTAGE OF OXYGEN AND CARBON DIOXIDE IN THE ATMOSPHERE OF A 2-COMPARTMENT WASTE DIGESTION UNIT DURING A PERIOD OF 10 DAYS

Days		Waste Chamber	Algae Chamber
1	CO2	0.07	0.032
	o <sub>2</sub>	19,84	20.39
3	co2	0.04	0.023
	$o_2^{-1}$	20.63	20.63
4	CO <sub>2</sub>	0.06	0.037
	02	20.63	20.52
6	co2	0.ü <del>4</del> 8	0.029
	02	20.62	20.73
10	CO2	0.03	0.018
	02	20.31	20.63

TABLE 9. GROWTH OF CHLORELLA IN TERMS OF PERCENT OF CONTROL ON COMPLETE KRATZ MEDIUM FORTIFIED WITH VARIOUS AMOUNTS OF UREA, URIC ACID, AND CREATININE

0.264%		1	175	157	138	111	122	133	140	150	132	132	117	124	116	110	110	114	110	106	106
Creatinine 0.066% 0.132%		•	182	131	125	104	116	127	137	138	123	126	111	115	111	106	107	108	107	102	101
		,	162	113	108	85	100	114	122	131	115	122	114	112	110	105	108	107	106	102	101
0.033%			145	118	100	111	26	111	110	119	104	105	104	100	96	26	26	95	94	91	33
0.096%			125	118	100	92	110	244	255	252	236	223	213	199	193	186	188	185	179	177	178
Uric Acid 24% 0.048%			125	100	200	207	506	188	176	185	180	169	164	150	147	145	145	142	140	138	135
Uric 0.024%			150	184	175	171	172	166	166	169	160	152	143	133	130	126	124	123	118	117	114
0.012%		•	237	210	180	185	181	181	176	176	169	156	146	134	136	128	127	126	121	122	117
Urea 0.5%		162	164	151	132	116	105	6	96												
N Deficient Kratz Medium			82	98	20	57	47	42	45	28	32	30	79	21	21	20	36	20	17	16	15
Davs	- 2 6 4	ιΩ	9	~	∞	6	10	11	12	13	14	15	16	17	18	19	70	21	22	23	<b>5</b> 4

TABLE 10. GROWTH OF CHLORELLA ON KRATZ MEDIUM IN WHICH NITRATE NITROGEN IS REPLACED WITH UREA, URIC ACID AND CREATININE ON A NITROGEN EQUIVALENT BASIS

Day	Kratz No. N	Urea 0.3032 g/1	Uric Acid 0.4243 g/l	Creatinine 0.3808 g/1
1				
2				
3				
4				
5				
6	82	137	607	82
7	86	184	684	100
8	50	138	550	63
9	57	140	492	71
10	47	128	487	53
11	42	131	458	55
12	45	135	433	56
13	28	128	409	52
14	32	127	379	50
15	30	128	361	44
16	26	118	318	38
17	21	110	285	33
18	21	113	280	34
19	20	106	259	31
20	22	108	247	32
21	20	109	244	30
22	17	104	225	27
23	16	104	222	2.6
24	15	101	213	25

ij જાં સ્ટ H Obio, Rpt. No. AMRL-TDR-62-142, A STUDY OF MICROBIOLOGICAL WASTE TREATMENT TECHNIQUES. Final report, November 1962, < ^ AFB, 6570th Aerospace Medical Research Laboratories, Wright-Patterson iv + 83 pp incl. tables, 24 refs. Aerospace Medical Division,

the water. On longer flights, the wastes either methods for the disposal of human wastes have duration (ca 100 to 1000 days) it would be most practical to treat the wastes with biocides and / ( over ) On space flights of moderate must be chemically or microbiologically oxistore them after dehydration for recovery of A number of chemical and microbiological chemically bound water could be dized so that the carbon and been surveyed.

equipment and the possible expenditure of some When used in conjunction with a photosynethetic An alternative to this procedure is suggested in used symbiotically for the conversion of wastes step, this might be the best solution to the prowhich algal and bacterial colonies separated by which methane-utilizing bacteria and algae are slower but takes place at a lower temperature. waste digestion and reutilization of the carbon. semipermeable membranes could be used for heat energy. Microbiological degradation is space voyages. A procedure is suggested by Sis. and blem for use on lunar colonies or prolonged complete but requires the use of elaborate recovered and used in photosynthe, Chemical oxidation is more rapid to protoplasm, with a minimum expenditure of energy.

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